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Subject: Project Assistance Completion Report for the Strategic Control of Grasshoppers and Locusts in Africa Project (USAID Project Officer: John Rifenbark, Grant #AOT-0517-G-00-4119-00)

I. SUMMARY PROJECT HISTORY

A. Introduction and Project Purpose

This project was authorized and obligated on June 17, 1994 as a grant in the amount of $332,049 to Montana State University (MSU) for research on strategic control of grasshoppers and locusts in Africa. Although the termination date was initially proposed for December 31, 1994, a modification provided a no-cost extension through September 30, 1995. Grant funding from USAID was made available to cover salaries, equipment, supplies, subcontracts, and other project expenses. In addition, the governments of Cape Verde and Mali provided the use of government facilities and contributed personnel during key periods of the project.

The purpose of the project, as stated in the proposal, was to develop environmentally sound, safe, effective, and cost-efficient alternatives to chemical pesticides for grasshopper/locust control in Africa. The overall goal was to help increase agricultural production, enhance food security, and decrease environmental and public-health risks associated with pest control. Specific objectives were the following:

- Develop the insect pathogen, *Beauveria bassiana*, for grasshopper/locust control in Africa and test it in large-scale field trials under actual grasshopper/locust control conditions.
- Further evaluate entomopoxviruses for long-term suppression of grasshopper/locust populations.
- Survey acridid populations in Cape Verde and/or Mali for indigenous entomopathogens with exceptional biocontrol potential.
- Strengthen the ability of the collaborating African institutions to undertake biocontrol research and conduct biocontrol operations.

The project objectives were achieved according to schedule with the exception of large scale field trials which were canceled in 1994 owing to low grasshopper densities in Mali resulting from weak rains and scarce hatching. Moreover, MSU and AELGA decided jointly to postpone large-scale trials anywhere until the following year owing to a possible problem with strain virulence which required further investigation. This postponement resulted in a no-cost extension until September 30, 1995, which permitted completion of large-scale trials according to the revised schedule.

By the end of the project in September 1995, all project goals were successfully met, the most important being the first-ever large-scale field testing of *Beauveria bassiana* in Africa. By contrast, the survey of indigenous pathogens did not lead to extensive screenings as expected. This resulted from a shift in priorities and resources required by extensive bioassay testing and virulence recovery efforts of the GHA strain at MSU. Moreover, further screening of indigenous pathogens in Mali would have been difficult owing to a lack of research cooperation and commitment by the Malian government. Shortly after the onset of the project, Malian project leaders began fighting over authority and equipment. As a result, project resources made available to them by MSU were not made available to scientists working on indigenous pathogen development. Collection, isolation, and storage of indigenous pathogens were nonetheless carried out by MSU and Mycotech in Montana.

B. Project Rationale

Promising research is underway in Africa which shows that deuteromycete fungi *Beauveria bassiana* and *Metarhizium sp.* can potentially be developed as biocontrol agents for grasshopper/locust control. Since 1989, this research has produced 80 to 90 percent mortality in laboratory and small-scale field trials against grasshoppers and locusts with several strains of deuteromycete fungi. By the end of 1993, US restrictions limiting the size of *Beauveria bassiana* field trials were lifted (following completion of toxicity tests and issuance of an experimental use permit by EPA), paving the way for large scale trials by MSU in Africa.

The need to test deuteromycete fungi on a large scale and ultimately develop these biocontrol agents for management of grasshopper and locust populations is underlined by several of the pathogen's key characteristics: (1) high infectivity and relatively rapid action; (2) feasibility of mass production; (3) ease of formulation and application with standard equipment; and (4) minimal regulatory requirements (the organisms are not genetically altered).

In addition to fungi, which are short-lived and require contact spraying, promising research is also underway on potential biocontrol agents that can be applied though natural inoculative mechanisms, thereby remaining active potentially for years. The most promising of these organisms is *Oedaleus senegalensis* entomopoxvirus. MSU trials in Cape Verde in 1991 with the virus observed: (1) mortality levels of around 50 percent within a week and 83 percent after 20 days in bioassays; and (2) in field trials indirect population-control effects such as weakened remaining individuals, nymphs prevented from reaching sexual maturity, decreased egg laying, and probable transfer to successive generations. Further research with more sophisticated testing equipment was needed to confirm the biocontrol potential of this entomopoxvirus.

There are several compelling reasons for using natural entomopathogens such as fungi and viruses for control of locusts and grasshoppers in Africa. Such organisms are adapted to attack a few closely related genera or species within a single insect family. Thus, they are highly target-specific. As a result, they are usually harmless to other living organisms and, unlike chemical pesticides, present few environmental risks. Most entomopathogens also present virtually no risks to human health. This characteristic makes them ideal for use in the developing world, where poor understanding of the toxicity of chemical pesticides often results in dangerous levels of human exposure and unacceptably high health risks.

C. Implementation

Preparation for large scale (100 hectare) field trials with *B. bassiana* in Cape Verde and Mali began in early-1994 with production of large quantities of the fungus, *B. bassiana* GHA, which was the only strain approved at the time by EPA for large scale trials in Africa. Unfortunately, that summer low grasshopper densities in Mali and poor field results in North America led MSU and AELGA to jointly postpone the large-scale field trials in Africa that year, to be completed in Mali or Cape Verde in 1995. To avoiding losing ground in other aspects of the research during the 1994 field season, the MSU team conducted small-scale field trials in Cape Verde with a different strain, *Metarhizium flavoviride* SP-9, the leading strain from Madagascar, and began field collections of indigenous pathogens in Mali and Cape Verde.

To test and improve GHA strain virulence, MSU and Mycotech spent the next year engaged in a comprehensive efficacy recovery program. Mycotech personnel conducted thorough evaluations of culture maintenance, production methods, and storage conditions. Steps to enhance strain virulence involved reisolation of GHA from infected grasshoppers. To evaluate the results, approximately 20 different bioassay experiments were conducted at MSU, each one lasting 10 days and typically involving from 150 to 200 grasshoppers, two or three different batches of GHA and multiple dosage levels of spores.

In July 1995, large-scale field trials were held in Mourdiah, Mali, involving personnel from MSU, Mycotech, Mali's Crop Protection Service (SNPV), and Mali's Institut d'Economie Rurale (IER). The purpose of these trials was to evaluate the biocontrol potential of *Beauveria bassiana* under "operational" field conditions. Specifically, these trials were designed to evaluate grasshopper *density reductions* in large, open field plots treated with *B. bassiana*.

The trials involved five different treatments: (1) *B. bassiana* GHA in a standard oil formulation, applied at a normal field dose rate. (2) *B. bassiana* GHA with a small amount of Dimilin (an insect growth regulator, or IGR) added as an enhancer. The amount of Dimilin added was a tenth of the normal field dose rate. (3) Dimilin alone, applied at a tenth the normal field dose rate, to determine the effect of this "enhancer" by itself. (4) Fenitrothion, applied at a normal field dose rate. Fenitrothion was selected as a standard, for comparison, because it is the chemical pesticide of choice for grasshopper control in Mali. (5) Untreated plots, for controls. The plot size used was 10 hectares, and there were three replications for each treatment. In sum, the field trial covered a total of 150 hectares. Density reduction was measured by a ring-count method developed at the Rangeland Insect Lab.

Preparation for entomopoxvirus evaluation trials began in mid-1994 with MSU researchers attempting to produce sufficient inoculum for trials. This attempt, however, was thwarted by unusually low densities of young grasshoppers in Montana susceptible to the virus. Subsequently, inoculum production activities were transferred to INIDA in Cape Verde using the project's colony of *O. senegalensis*. The production work was successful, but completed too late for the 1994 field season, and was rescheduled for the next field season.

In late August 1995, small-scale field trials with *Oedaleus* entomopoxvirus were conducted on Santiago Island in Cape Verde. These trials were led by Maria Lobo-Lima, MSU's research coordinator for Africa, and involved personnel from Cape Verde's Crop Protection Service and INIDA (the national agricultural research institute). The trials consisted of treating grasshoppers in eight $50m^2$ low-walled, uncovered enclosures constructed of wooden stakes

and mosquito netting. Four enclosures were treated with entomopoxvirus, and four were left untreated, as controls. Following treatments, 325 grasshoppers were collected from each enclosure (a total of 2,600) and taken to the laboratory at INIDA to monitor for infection. Monitoring lasted for 30 days.

Surveys for indigenous pathogens in Mali began in July 1994, when MSU/Mycotech researchers extensively surveyed the grasshopper population in the Mourdiah area, collecting some 30 sporulating cadavers for further research. Over the course of the next year, some 200 additional infected grasshoppers were sent by Malian IER researchers to Montana, retaining half of the cadavers for their own research and inventory. Single spore isolations were completed on a limited number of the specimens in Montana, while the remainder were labeled and prepared for storage. A matching inventory was prepared in Mali. In Cape Verde, in collaboration with INIDA personnel, MSU surveyed the local population of *Oedaleus senegalensis* for new endemic pathogens.

Integral to the project were MSU efforts to **strengthen the capacity of collaborating African Institutions** so they could undertake biocontrol research efforts and actual biocontrol operations. Beginning in 1994, Malian researchers Dr. Ousmane Cisse and IER technician Mariam Diakite were trained by MSU scientist Delgado in handling and evaluation of entomopathogenic fungi from grasshoppers. These two individuals were chosen for training because previously trained Malian personnel (including two Ph.D.s) were away on leave to Paris and the U.S., and would no longer be available to the project. Training of Malians included (1) isolation of *B. bassiana* and other fungi from dead grasshoppers, soil, and vegetation, (2) identification of different fungi and preparation of culture media, and (3) standard procedures for bioassays with fungi against grasshoppers. In early 1995, a review session was held in Bamako covering the same techniques. Later in 1995, IER scientists and SNPV field technicians were trained in field trial design, layout, and implementation for large-scale field trials. In Cape Verde, training of INIDA personnel was ongoing, facilitated by the work program of MSU contract scientist Lobo-Lima, which included laboratory and field work on entomopoxvirus, and field surveys for new pathogens.

II. PROJECT OBJECTIVES AND ACCOMPLISHMENTS

This section provides a summary of major project objectives and the accomplishment of each objective.

1. Further evaluate and develop *B. bassiana* as a biocontrol agent for grasshopper and locust control in Africa, particularly through large-scale field trials.

Large-scale field trials were successfully carried out in Mourdiah, Mali, in July 1995 by MSU, Mycotech, Mali's SNPV and Mali's IER. The fungal agent tested was the only fungal agent approved by EPA for large-scale testing in Africa at the time, *Beauveria bassiana* GHA.

Results from the 1995 trials in Mali were better than expected, given GHA's unexpectedly poor performance in 1994 North American large-scale trials. Virulence recovery efforts during the course of the year had obviously paid off. But results were still below efficacy levels achieved previously in Africa with GHA during small scale trials and bioassays. Fortunately, significant grasshopper population reductions *did occur*, both using fungi alone and formulated

with an additive, and these results are expected to improve as MSU's more potent strains (including one isolated from Madagascar) are approved for large-scale testing and use.

Changes in grasshopper populations in Mali (adjusted for control mortality):

Treatment	Ave. Daily Survival Rate (DSR)	Adjusted Control
B. bassiana	0.97	40 %
Dimilin (low dose)	0.97	33 %
B. bassiana + Dimilin (low dose)	0.94	58 %
Fenitrothion	N.A.	73 %

The density reduction in plots treated with *Beauveria bassiana* alone was significant at a very slow rate. The density reduction in plots treated with Dimilin alone was less important and slower. However, the density reduction in plots treated with *Beauveria bassiana* plus Dimilin was very significant and relatively rapid. The predicted combined effect of the *Beauveria bassiana* and the Dimilin treatment based on their action alone was not different from the effect observed with the treatment when they were added up. It was concluded that *Beauveria bassiana* used at the recommended field rate with Dimilin used at a sub-lethal dose level (one-tenth of the recommended field rate) had an additive effect on grasshopper populations in field plots.

The number of grasshoppers in treated plots decreased significantly over the entire two-week period (adjusted for control mortality) in most cases. The exceptions occurred in fenitrothion-treated plots where the population decreased sharply in the first 48 hours and then began to rise again. This effect could be due to the elimination of natural predators and parasites, however this theory was not tested at the time.

The results show that biocontrol of grasshoppers can be achieved on a large scale using even a relatively weak strain of fungus, and that this effect can be enhanced with low doses of a narrow spectrum additive such as Dimilin. Moreover, this study raises questions about the medium-term efficacy of chemical pesticides which cannot apparently sustain the level of control demonstrated in the first couple of days after treatment.

2. Evaluate entomopoxviruses as a long-term population suppression agent. Field trials using the *O. senegalensis* entomopoxvirus were carried out in August 1995, involving treatment of grasshoppers in eight 50 m^2, low-walled, uncovered enclosures. Unfortunately, high infection rates in both treated and untreated plots from a naturally occurring epizootic of *Metarhizium* obscured results of the entomopoxvirus. Mortality in both treated an untreated groups ranged from 62 to 77%, mainly due to fungal infection. Infectivity from *Oedaleus* entomopoxvirus ranged from 41% to 44% in grasshoppers collected 24 and 48 hours after treatment. Grasshoppers surviving the 20 day experiment had a low rate of infection (about 4%). However, levels of infectivity would have certainly been much higher had it not been for the fungal infection.

3. Survey acridid populations in Cape Verde and/or Mali for indigenous entomopathogens with exceptional biocontrol potential.

From initial surveys in Mali and from subsequent collections by IER, the number of infected grasshoppers available to researchers reached more than 200, of which 15 were prepared as single spore isolations. Fungal pathogens isolated included *Metarhizium sp.* and *Beauveria bassiana*. Further evaluation of these isolates was not done owing to the higher priority of conducting bioassays with the approved *B. bassiana* GHA strain. Moreover, it was feared that Malian strains stood little chance of development without greater commitment and cooperation from the Malians.

Survey work in Cape Verde for new endemic pathogens led to the discovery of a dozen different fungal isolates and two new species of microsporidia. Infected grasshoppers and single spore isolations from Cape Verde and Mali are currently in storage at Mycotech and in their countries of origin.

4. Strengthen the ability of the collaborating African institutions to undertake biocontrol research and actual biocontrol operations.

Training and capacity building in Mali met with mixed success in this and previous grant work undertaken in that country. Without exception, Malian scientists, technicians and administrators showed a keen interest in starting up the project and participating in training and field events. However, their fundamental interest and commitment to implementation of the project, was lacking. It was extremely difficult to overcome this problem no matter the commitment by MSU.

On balance, the Malians were eventually capable of carrying out all of the field and research tasks of the project under MSU direction, but were lacking leadership and direction. In evidence, the successful large-scale field trials which were carried out with MSU supervision, were done with great difficulty. Malian participation included plot layout, testing of spore viability, spraying, sampling for density reductions through the use of ring counts, and data recording. The problem for MSU was that the Malians who showed up for the field trials were not the same ones as previously trained, forcing MSU to repeat previous training and to work with all new personnel at the last minute. Similarly at IER, the original research team intensively trained in Bozeman for the development of indigenous pathogens was no longer available once the project began. Two of the trained scientists left unexpectedly on 2+ year educational and pregnancy leaves, and a third moved into administration. Thus, MSU had to begin training Malian replacements, one of who was a contractor and had no institutional pull once an internal Malian struggle for resources began. The other was a relatively low-level technician, who was enthusiastic but without leadership skills.

In Cape Verde, the picture was completely different. Here capacity-building was so complete that Cape Verde's personnel began training personnel from other African countries both in simple procedures and complex programs for developing indigenous pathogens. And although two of the three MSU-trained personnel from INIDA eventually took other jobs, two remain in Cape Verde, and one remains at INIDA where research on biocontrol of grasshoppers continues. As a result of MSU's work with INIDA, Cape Verde has made important contributions to development of the technology and will be prepared to implement biocontrol of grasshoppers at home when products becomes commercially available.

III. PROGRESS TOWARD ACHIEVEMENT OF PROJECT PURPOSE

The purpose of the project was to develop environmentally sound, safe, effective, and cost-efficient alternatives to chemical pesticides for grasshopper/locust control in Africa. Insofar as the main project activities permitted, important progress was made toward the project purpose. Notably, successful large scale field trials were carried out with a safe (EPA experimental use-permitted), cost-effective (about the same price as chemicals) biocontrol agent. These large-scale trials with *Beauveria bassiana* were the first ever held in Africa. They demonstrated the feasibility of biocontrol technology for use under African conditions and carried out by African personnel. They identified an additive for improving the efficacy and kill speed of fungi, and they raised questions about the sustained efficacy of chemicals. For although the short term efficacy of *B. bassiana* GHA was less than chemicals, the effect was longer lasting. Research and development efforts currently underway at MSU and other organizations are expected to eliminate the short-term efficacy gap and explain the difference in long term effects between fungi and chemicals.

IV. SUMMARY OF LESSONS LEARNED

Several lessons have been learned from this project, the most important one being that USAID and implementing agencies are on the right track in developing fungi-based biocontrol agents which are safe, effective, and manageable in Africa. The project's large-scale demonstration trials showed that fungus can be an effective biocontrol agent. The trials also improved our understanding of what efficacy means. MSU now knows that although *Beauveria bassiana* GHA achieves respectable results, (1) it must push ahead with more virulent strains (such as a promising new *Metarhizium* from Madagascar) for regional development, (2) it must continue doing research on additives, and (3) it must compare fungi and chemical control in a longer time frame. In planning future large-scale trials, MSU recognizes the importance of having both untreated control plots and control plots treated with a chemical agent.

Other evidence supporting the development of fungal biocontrol agents was found quite unexpectedly in trying to measure the effects of an entomopoxvirus on grasshoppers. The effects of the manually applied virus was mostly obscured by a naturally occurring epizootic of *Metarhizium* in the region of the field trials in Cape Verde. Such naturally occurring epizootics are the type of phenomenon which biotechnology of this sort attempts to emulate or augment.

A number of lessons were learned about capacity building through MSU's work in Mali. The most important lesson for the future is to assess the level of commitment and the time horizon of project participants, especially the project leader proposed by the government. Both in the training of Malian IER and SNPV personnel, MSU efforts were undermined when trained personnel were subsequently transferred, hired away, or did not get access to project resources in a way that led to sustainable work. The result was an ongoing training of new faces, misallocation of equipment, and a lack of continuity and commitment to the work. This problem was exacerbated by the small number of qualified personnel in Mali, the multiple demands on their time (qualified people were transferred as a means of generating new projects to obtain donor resources), and a general cynicism among Malian project leaders about donor aid. Continuity of the work in Mali was also interrupted prior to the beginning of the project, owing to an interruption in A.I.D. funding, which may have contributed to the turnover of Malian personnel. By contrast, in Cape Verde, the high level of personal, professional and institutional commitment, led to a positive and highly productive project in that country.